FISHERY RESEARCH

WHIRLING DISEASE RESEARCH REPORT: SEASONAL INFECTIVITY OF *MYXOBOLUS CEREBRALIS* TO RAINBOW TROUT *ONCORHYNCHUS MYKISS* EXPOSED AT MONTHLY INTERVALS IN THE SOUTH FORK BOISE RIVER

ANNUAL PROGRESS REPORT
March 30, 1998 to April 22, 1999

Prepared by:

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Eagle Fish Health Laboratory

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ABSTRACT

We conducted a field trial in the Lower South Fork of the Boise River in southwest Idaho to determine if there were detectable seasonal differences in infectivity of the exotic parasite *Myxobolus cerebralis*, the causative agent of salmonid whirling disease. Juvenile rainbow trout *Oncorhynchus mykiss* were exposed for 10 days to river water at approximately monthly intervals from March 30, 1998 to April 22, 1999, and then reared on specific pathogen-free water at the Eagle Fish Health Laboratory for a sufficient period for the parasite to fully develop to the spore stage. Prevalence within each exposure group and individual levels of infection (intensity) were determined in the laboratory using the pepsin/trypsin digestion spore enumeration method. In general, both prevalence and intensity were greater when mean water temperatures exceeded 6°C, but prevalence was greatest when temperatures were rising in the spring, while intensity was greatest when temperatures had peaked and begun to decline in the fall. Individual levels of infection were reduced when exposure water temperatures averaged <9.0°C but were never eliminated, even when the mean water temperature was as low as 2.6°C. There were individual instances when sudden increases in total water flow or velocity may have been related to statistically significant decreases in prevalence or intensity of infection, but more gradual flow changes did not seem to impact infectivity. The distribution of spore counts from individual fish was significantly lowest in the group exposed March 2-12, 1999 and significantly greatest in the group exposed September 28–October 8, 1998 ($\alpha = 0.05$). Evidence suggests that water temperature was the primary factor involved with both of these significant findings.

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INTRODUCTION

Myxobolus cerebralis (phylum *Myxozoa*, class *Myxosporean)* is the causative agent of whirling disease in salmonid fishes (Hoffman 1999). The parasite has a complex lifecycle involving an intermediate oligochaete worm host, *Tubifex tubifex*. The worms release a triactinomyxon (TAM) stage that is infectious to salmonid fishes. The parasite attacks the cartilage of the fish and causes clinical signs of disease, the intensity of which is directly proportional to the number of parasites infecting the individual host. Spores develop and are released to the environment when the host fish dies and decomposes or is eaten and digested by a predator. Spores are then ingested by *T. tubifex* to complete the cycle (Hedrick and El-Matbouli 2002). The rainbow trout *Oncorhynchus mykiss* has been shown to be one of the most susceptible species to clinical whirling disease (Hedrick et al. 1999), and the presence of the parasite has been implicated in the decline of numerous wild rainbow trout populations (Nehring and Walker 1996; Vincent 1996)

The presence of *M. cerebralis* has been documented in naturally produced rainbow trout from the South Fork of the Boise River below Anderson Ranch Dam (Elle 1998). Observations from this single mid-summer exposure indicated that levels of infection in individual fishes ranged from none detected to high, with no clinical signs of disease observed. Prevalence of infection within the river has been demonstrated to progressively increase as distance downstream from the dam increases (Hiner et al. 2001). The site for the current study was located in an area of intermediate prevalence. This study was designed to compare the prevalence and intensity of *M. cerebralis* infections in groups of similar juvenile rainbow trout exposed in the South Fork of the Boise River at approximately monthly intervals over the course of a full year.

OBJECTIVE

The objective of this study was to increase our understanding of changes in infectivity by *M. cerebralis* as related to the seasonal changes in water temperature and flow in a river environment. Additional benefits included the opportunity to evaluate exposure and laboratory methods and gather baseline information for future field studies.

STUDY SITE

The exposure site was in the South Fork of the Boise River (Figure 1), approximately 18.7 km downstream of Anderson Ranch Dam (Elmore County, Idaho; UTM 11, 615879E, 4806785N). The actual location was near the south bank, approximately 200 m below the Danskin Bridge, identical to that of Elle (1998). The U.S. Bureau of Reclamation controls water discharge from the dam and provided the daily water flow data for this study. No flow measurements were available for the few tributary streams between the dam and the study site. However, none of these streams are large, so their contribution to total river volume was considered negligible. Following exposure in the river, the fish were held in the wet laboratory at the Idaho Department of Fish and Game's Eagle Fish Health Laboratory in Eagle, Idaho, where they were subsequently sampled and tested for the presence of *M. cerebralis*.

METHODS

Experimental animals were hatchery-produced juvenile rainbow trout. In order to expose fish of similar age and size during every month of the year, it was necessary to use different lots and strains of rainbow trout fry, as available. The majority of the exposure groups were Hayspur- or Troutlodge-strain rainbow trout. One exception was a group of spring-spawning Colorado River-strain fry from Hayspur Hatchery (Group VI). All fish were age-0+, and mean individual weight and length at exposure for the different groups ranged from 0.4 to 4.5 g and from 33 to 74 mm.

Test groups of approximately 50 fish each were placed in a single, cylindrical, aluminum live box, 0.45 m long by 0.3 m diameter, and held in the river for periods of ten days each. A notable exception was Group VI, which had over 1000 fish and was exposed for seven days in order to cooperate with a Fumagillin study conducted by personnel from the U.S. Fish and Wildlife Service. Water temperatures during the test periods were recorded hourly using a StowAway® XTI temperature logger placed inside the box. The StowAway® was left in the river between exposure groups to continually monitor water temperatures. After exposure, the fish were transported to the Eagle Fish Health Laboratory and held in experimental tanks on specific pathogen-free well water at constant 13°C until they had accumulated a minimum 1800 Celsius temperature units (CTU). The fish were then sacrificed. Whole heads from 20 individuals per group were randomly selected and processed for spore enumeration using a modified version of the pepsin/trypsin digest (PTD) method (Markiw and Wolf 1974). Intensity of infection was determined for each sample by placing a drop of the PTD suspension on a hemocytometer and counting the number of spores observed in the grid area. If no spores were observed within the grid, but at least one spore was seen outside the grid, the individual was considered to be positive and given an arbitrary spore count value of 0.5 (half of minimum sensitivity of the test). Total number of spores per fish head was estimated by multiplying the actual count by 1667 (Markiw and Wolf 1974). Intensity for the group was then determined by averaging the estimated individual spores per head for all individuals. Prevalence of infection was determined as the percentage of individuals within a sample group from which spores were detected. Excess fish from selected lots were tested by polymerase chain reaction (Andree et al. 1998) or examined by histology to confirm that the spores detected by PTD were indeed *M. cerebralis* and not a different *Myxobolus* species.

The Wilcoxon Rank Sum Test (WRST) was used to test if the distribution of spores detected from individuals in pairs of exposure groups were significantly different ($\alpha = 0.05$). The WRST is a nonparametric procedure to test if two populations are identical, analogous to a single-tailed *t* test but using an ordinal scale. Nonparametric statistics were necessary because of the different sources for the exposure populations, and WRST was chosen because both prevalence and intensity were reflected in the ordinal values. In addition, WRST does not require equal sample sizes, which became beneficial when eight of 20 samples from Group I were lost in the laboratory. Linear regression analysis was performed to demonstrate relationships between prevalence and intensity, prevalence and mean water temperatures and flows during exposure, and between intensity and mean water temperatures and flows during exposure.

RESULTS

Twelve groups of fish were exposed in the river from March 30, 1998 to April 22, 1999 (Table 1). Spores were detected by PTD in at least one individual from every exposure group. Histology revealed spores in cartilage from one of two excess fish from Group II, and PCR tests confirmed *M. cerebralis* DNA in individuals from Groups II, IX, and XI. No mortalities attributable to whirling disease occurred during the rearing periods. Subacute signs of infection, primarily deformities of the head and opercles, were common at sampling. Only two fish from Group VI, out of the 1,300+ total fish involved in this study, showed black tail or whirling behavior.

Detected prevalence of infection was 80% or greater from late April through November (Table 2), with the exception of the September group (VI) that may have been more influenced by experimental variables than by the environment. Mean daily water temperatures during this period averaged 9.4°C (range 5.8°C to 16.7°C), while water flows peaked at >100 m^3/s then gradually decreased to the seasonal minimum of about 9 m^3/s (Figure 2). Prevalence was greatest in May, June, and July (100%, 95%, and 100%, respectively). The greatest intensity of infection was detected in Group VII, exposed in early October (49,500 mean estimated spores per head; prevalence 90%). Wilcoxon's RST results indicated the distribution of numbers of spores detected from individuals in Group VII was significantly different from all other groups (P-value range: <0.050 to <0.001). The highest mean daily water temperatures (range 12°C to 15°C) were recorded about three weeks prior to the Group VII exposure, and the lowest water discharges from Anderson Ranch Dam (9 m^3/s) were implemented at the same time.

Prevalence and intensity of infection in Group V (August) were both less than in Group IV (80% v. 100% and 3.5 v. 9.2 mean spores per head, respectively). Wilcoxon's RST indicated a significant difference (0.005>P>0.005) between the distribution of numbers of spores detected in these two groups.

The distribution of numbers of spores detected in Group V was also significantly less than those for Groups VII (October; P<0.0005) and VIII (November; 0.005>P>0.001). This disregards Group VI, which will be dealt with separately because of numerous experimental variables. The increase in prevalence between the groups was only 10% (from 80% in Group V to 90% in both Groups VII and VIII), but differences in individual intensity of infection between these sets of observations were marked (nearly 8-fold between Group V and Group VII).

Prevalence and intensity were both lowest in Group XI (March 1999; 5% and 170 mean estimated spores per head, respectively), and the distribution of numbers of spores detected for Group XI was significantly different (WRST) from all other groups (P-value range: <0.025 to <0.0005). Group XI was exposed one month after the minimum water temperature was recorded for the season and less than a month after water flows rapidly increased (Figure 2). Excess fish from Group XI tested positive for *M. cerebralis* DNA by PCR, indicating that prevalence may have been higher if a test more sensitive than PTD had been used. Group X experienced the lowest mean water temperature during exposure (2.6°C), yet had a prevalence of 70%, and individual levels of infection averaged 7,250 spores/head. Water flows had been consistently low for several months before and during the Group X exposure.

A rapid increase in water flow was also observed in June 1998 during the Group III exposure period. The Wilcoxon RST test results indicate that the distribution of numbers of spores detected from individuals in Group III was significantly different than those of both Groups II (0.010>P>0.005) and IV (0.0010>P>0.0005). Prevalence in Group III was 95%,

compared to 100% in both Groups II and IV. However, the intensity of infection in Group III was less than half of that of Groups II or IV (6,210 mean estimated spores per head compared to 13,330 and 15,250, respectively).

Additionally, WRST analysis of Group XII indicated that the distribution of spores was significantly different (P-value range: <0.0250 to <0.0005) from all other groups with the exceptions of Groups I and VI (P >0.050). It would be expected that Groups I and XII would not be different, as they were both exposed at approximately the same time of year (March 30 to April 9, 1998 for Group I, and April 12 to 22, 1999 for Group XII) and the environmental conditions of flow and water temperature were similar (Table 1). Experimental deviations for Group VI may explain the lack of difference for that pairing. The difference between Groups XI and XII (0.025>P>0.010) is notable in that intensity of infection increased from 170 to 1,920 estimated mean spores per head and prevalence increased from 5% to 30%, during a period when the mean water temperature increased from 3.7°C to 5.5°C and mean river flow decreased from 60 m^3/s to 51 m^3/s.

Linear regression analysis indicated no significant relationships of prevalence with mean water temperature during exposure (P = 0.1463, R^2 = 0.1988), with mean flow during exposure (P = 0.9559, R^2 = 0.0003), or with mean initial fish weight (P = 0.6520, R^2 = 0.0211). Similarly, there were no significant relationships of mean number of spores per head in each group with mean temperature (P = 0.2742, R^2 = 0.1180), with mean flow (P = 0.1523, R^2 = 0.1936), or with mean initial weight (P = 0.4676, R^2 = 0.0594). There was a significant relationship of prevalence with total CTUs during exposure (P = 0.0410, R^2 = 0.3547), but there was not a significant relationship between mean number of spores per head and CTUs (P = 0.1882, R^2 = 0.1663).

During a period of 147 days from September 17, 1998 to February 11, 1999, the water discharge from Anderson Ranch Dam remained an almost constant 9 m^3/s, while the mean water temperatures declined at a very consistent rate (Figure 2). Linear regression analysis for only the four groups exposed during this period indicated significant relationships of mean spores per head with both mean water temperature (P = 0.0410, R^2 = 0.9590), and with total CTUs during exposure (P = 0.0340, R^2 = 0.9217), although the relationships of prevalence with mean water temperature and total CTUs were not significant (P = 0.1151, R^2 = 0.7831, and P = 0.1001, R^2 = 0.8098, respectively).

As mentioned above, Group VI was comprised of fish from a different stock than all other groups, contained many more fish with smaller average size than any of the other groups, and had less exposure time (7 days v. 10 days). Group VI experienced the highest mean daily water temperature of all groups (11.7°C), but accumulated fewer CTUs during exposure due to the shorter duration. This is the reason for the disparity between the linear regression analysis for prevalence and mean temperature (P = 0.1463, R^2 = 0.1988), and the analysis for prevalence and total CTUs during exposure (P = 0.0410, R^2 = 0.3547). If the analyses were done disregarding the data from Group VI, the relationship between prevalence and mean temperature would be significant (P = 0.0338, R^2 = 0.4098), while that between prevalence and total CTUs during exposure would remain significant (P = 0.0294, R^2 = 0.4263). Prevalence was much lower in Group VI (45%) than in Groups V and VII (80% and 90%, respectively). Intensity of infection was slightly higher in Group VI than in Group V (6250 v. 5880 estimated mean spores per head, respectively), but much less than in Group VII (49,500). The WRST results indicated that differences in the distributions of detected numbers of spores between Group V and Group VI were not significant (P >0.05), but the differences between Group VI and Group VII were significant (P <0.0005).

DISCUSSION

The primary objective of the study was met by determining that there were significant seasonal variations in *M. cerebralis* infectivity in a natural river environment. Vincent (1998) reported that the intensity of *M. cerebralis* infections was strongly correlated with water temperatures (e.g., with low severity at low temperature, greater severity beginning at about 9°C, and peak severity at about 14°C). This study supports those conclusions while further demonstrating that infectivity persists in a natural environment when temperatures average between 2°C and 3°C. With the exception of the September group (VI), which was affected by a number of other variables, prevalence of infection was ≥80% from May through November 1998. This entire period coincided with mean water temperatures >6°C and daily peaks >10°C. Prevalence remained at 65-70% in December and January, when daily peaks in water temperature still reached 5-7°C although the mean temperatures were much colder. The only time when prevalence was markedly reduced was in early March 1999 following three months of mean water temperatures <4°C. This suggests that the *T. tubifex* worms continued to shed TAM stages of the parasite for some time after mean temperatures decreased below optimum levels. Perhaps the TAMs developed during the warmer months, persisted in the worm host, and were shed when daily temperature fluctuations briefly reached more optimal levels.

Periods of high prevalence (90–100%) coincided with both the highest and lowest river flows. Water flow may have had an impact on prevalence during two periods of rapid increase in discharge from Anderson Ranch Dam. In June 1998, when temperatures were near optimal for infection but flows had more than doubled from the previous exposure period, prevalence dropped 5% and intensity of infection was reduced by more than 50%. The most dramatic example was between January and March 1999 (Groups X and XI), following four months of the coldest water temperatures of the year. Flow rate increased almost 7-fold (from 308 ft^3/s to 2,117 ft^3/s). Prevalence dropped from 70% to 5%, and intensity declined from 7,250 to 170 mean estimated spores per head (almost 98%). However, infectivity rebounded one month later (Group XII, prevalence 30%, 1,920 mean estimated spores per head). The mean water temperature between Groups XI and XII increased 1.8°C, while the mean river flow experienced only a moderate decrease of 308 cfs (Table 1).

Intensity of infection was greatest in the sentinel groups exposed in October and November, shortly after the highest water temperatures of the year and right after the lowest flow rates were initiated. This is again consistent with Vincent (1998), who reported greatest severity between 9°C and 14°C, and with the assumption that reduced water flows concentrate the infective TAMs. Prevalence in October was 90%, with a range of individual intensities from zero to 180,000 estimated spores per head. It is interesting to note that no spores were detected by PTD from two of 20 fish in the sample, although the assumption was made that each individual fish in the live box would have the same probability of becoming infected. It is possible that they were infected below our detection level, yet their level of infection was substantially different from those individuals with the highest intensity. Two possible answers for this situation may be hypothesized. First, some individual fish within a given population may have greater inherent resistance to *M. cerebralis* infection or spore development. Second, water flow patterns within the live box may not have created a totally random distribution of TAMs, and behavioral differences between individual fishes may have resulted in different risk of physical contact with the TAMs.

An unexplained deviation in the data is Group V (August), when the distribution of detected spores was significantly less than the previous month, even though water temperature was in the optimum range and water flow was steadily declining. A weakness in this trial is the lack of replicated data sets that would confirm whether such an anomaly truly reflected what was happening or was just a random event.

Results from this study suggest that water temperature is the dominant factor in *M. cerebralis* infectivity, although rapid increases in total river flow were shown to have an effect. Possible explanations include the dilution of a finite number of TAMs in the greater water volume, or the inhibition of an individual TAM's physical ability to attach to a fish due to increased water velocity. Some managers have suggested that introducing a stock of rainbow trout that spawns at a different time may reduce the impact of whirling disease by allowing emerging juveniles to avoid periods of highest infectivity. The information from this trial suggests that such a strategy would not be effective in this particular river environment, because the period during which infectivity is significantly reduced is not long enough. Trout growth and development are directly regulated by water temperature. If low temperature is the major factor in reduced infectivity, it is unlikely that juvenile fish could outgrow the most susceptible life stage during the limited period of reduced infectivity.

In addition, this study gave us the opportunity to evaluate and perfect exposure methods and laboratory techniques that have been used for subsequent trials in the Salmon River, Pahsimeroi River, Lemhi River, and Teton River.

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Table 1. Summary of data collected during the exposure of juvenile rainbow trout to *Myxobolus cerebralis*-positive waters of the South Fork of the Boise River, Elmore County, Idaho. Exposure periods were of ten-day duration from March 1998 through April 1999 at a site below Danskin Bridge (UTM 11, 615879E, 4806785N).

Group	Exposure Dates	Exposure Water Temperature (°C)		Total CTUs During Exposure	Total CTUs at Sampling	Mean River Flow During Exposure (m^3/s)[a]	Mean Fish Size at Exposure (g/mm)
		Mean	Minimum / Maximum				
I	3/30 — 4/9/98	4.9	3.5/7.2	48.4	1878	46	4.5/74
II	4/28 — 5/8/98	6.5	4.6/10.2	64.9	1856	41	0.9/43
III	5/28 — 6/8/98	9.5	7.9/11.7	104.5	1886	101	3.6/69
IV	6/24 — 7/4/98	11.2	9.2/13.6	112.6	2004	71	2.0/57
V	8/7 — 8/17/98	10.1	8.5/12.5	100.5	1879	45	1.4/51
VI	9/3 — 9/10/98[b]	11.7	8.9/15.1	82.3	1911	17	0.4/33
VII	9/28 — 10/8/98	10.2	6.5/14.0	102.8	1984	9	1.0/45
VIII	10/27 — 11/6/98	7.1	3.5/12.5	71.4	1953	9	1.3/49
IX	12/4 — 12/14/98	3.8	0.3/7.4	36.4	1842	9	0.8/42
X	1/14 — 1/24/99	2.6	-0.2/5.2	31.4	1860	9	1.0/46
XI	3/2 — 3/12/99	3.7	2.6/5.4	37.4	1900	60	1.2/48
XII	4/12 — 4/22/99	5.5	4.0/7.9	55.6	1972	51	1.3/50

[a] Mean daily discharge in cubic feet per second from Anderson Ranch Dam during the exposure period (U.S. Bureau of Reclamation, Snake River Area Office, Boise, Idaho).

[b] Seven-day exposure to coincide with a USFWS fumagillin trial.

Table 2. Summary of prevalence and intensity of infections of juvenile rainbow trout exposed to *Myxobolus cerebralis*-positive waters of the South Fork of the Boise River, Elmore County, Idaho, following ten-day exposure periods from March 1998 through April 1999.

Group	First Day 0f Exposure	Sample N	Prevalence (Percent Infected)	Mean Spore Count Per Head (pepsin / trypsin digest)	Mean Estimated Spores Per Head	Range of Estimated Spores Per Head (x1000)	Group[a]: II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Group
I	Mar 30	12	58	2.6	4,300	0—20.0	S	N	S	N	N	S	S	N	N	S	N	I
II	Apr 28	20	100	8.0	13,330	1.7—40.0		S	N	S	S	S	N	N	S	S	S	II
III	May 28	20	95	3.7	6,210	0—31.7			S	N	S	S	S	N	N	S	S	III
IV	Jun 24	20	100	9.2	15,250	1.7—45.0				S	S	S	N	N	S	S	S	IV
V	Aug 7	20	80	3.5	5,880	0—26.7					N	S	S	N	N	S	S	V
VI	Sep 3	20	45	3.8	6,250	0—38.3						S	S	S	N	S	N	VI
VII	Sep 28	20	90	29.7	49,500	0—180.0							S	S	S	S	S	VII
VIII	Oct 27	20	90	12.8	21,420	0—68.3								N	S	S	S	VIII
IX	Dec 4	20	65	8.0	13,330	0—46.7									N	S	S	IX
X	Jan 14	20	70	4.4	7,250	0—33.3										S	S	X
XI	Mar 2	20	5	0.1	170	0—3.33											S	XI
XII	Apr 12	20	30	1.2	1,920	0—15.0												

[a] The distributions of individual spore counts in paired groups were tested for significant difference using Wilcoxon's Rank Sum test. N = not significantly different. S = significantly different, α=0.05.

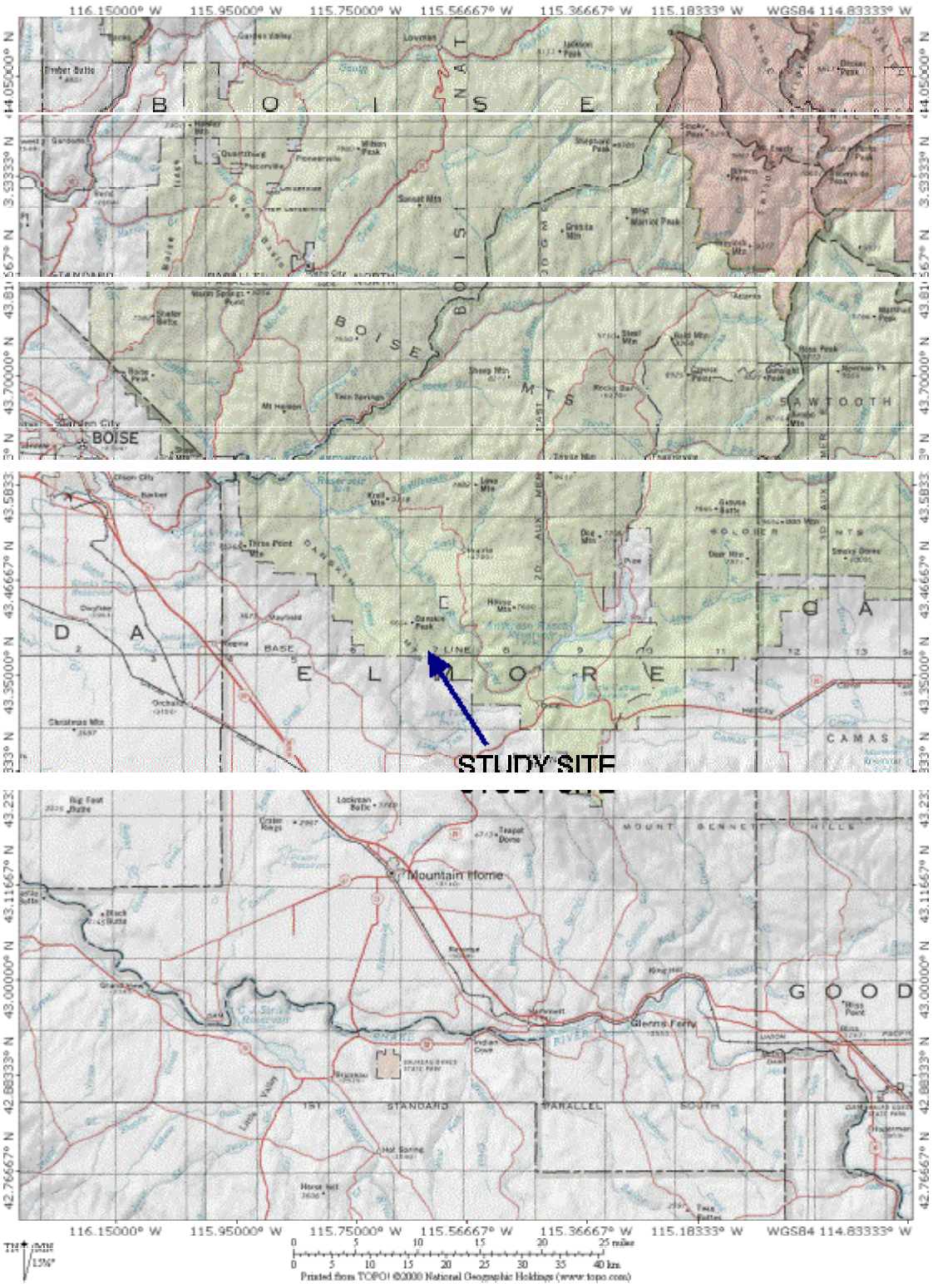

Figure 1. Topographic map detailing the study site of an Idaho Department of Fish and Game clinical field trial exposing rainbow trout *Oncorhynchus mykiss* fry to the waters of the South Fork Boise River, March 30, 1998 to April 22, 1999. UTM 11, 615879E, 4806785N.

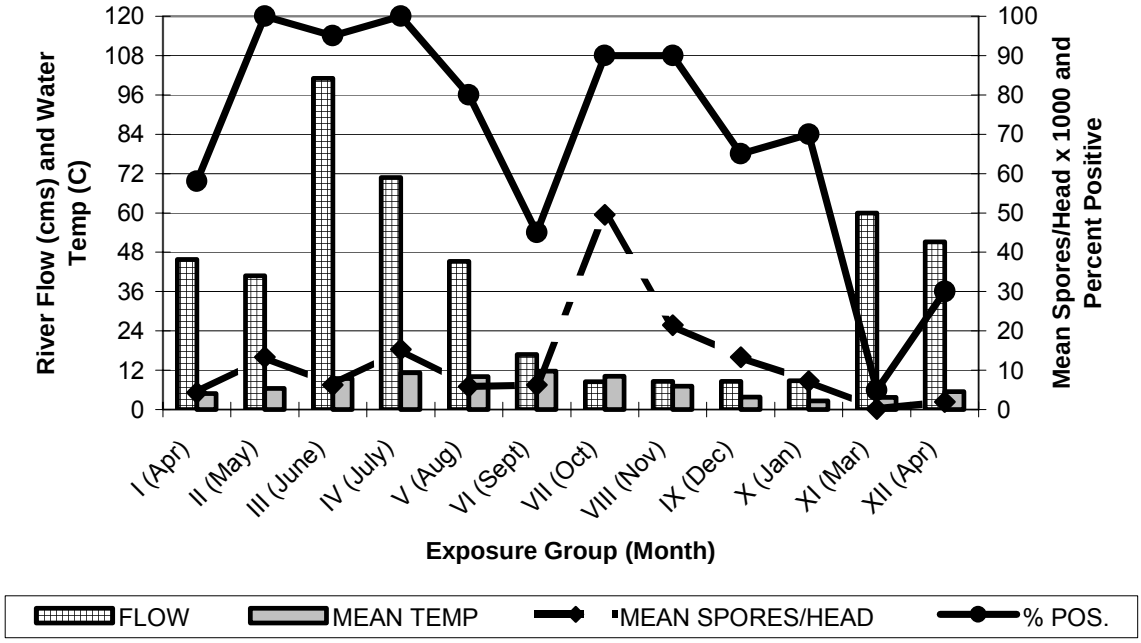

Figure 2. Mean flow rates and water temperatures in the South Fork of the Boise River during 12* ten-day periods from March 30, 1998 to April 22, 1999, and the corresponding prevalence (% infection) and mean individual intensities (spores/head) of *Myxobolus cerebralis* from groups of rainbow trout juveniles exposed to the river environment for the same 12 periods. (*Period VI was for only 7 days).

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